SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

SOUTH. BRAZ. J. CHEM., Vol.3 N° 3, 1995

DETERMINATION OF ACETATE IN FOODS BY ION CHROMATOGRAPHY–FLOW INJECTION

*E. Esteve–Juan, R. Puchades and A. Maquieira**
Department of Chemistry, Polytechnic University of Valencia,
E–46071 Valencia, Spain.

ABSTRACT. A method for acetate determination using IC separation and FI post–column spectrophotometric detection based on the $La(OH)_3$–I_2 reaction has been developed. The chromatographic conditions (eluants, flow–rates, sensitivity and so on) and FI variables (length of coils, injection volume, pH,...) were optimised. Linearity was observed over the concentration range 0 to 1 gl^{-1} with HAc as standard (r = 0.9916) and a detection limit of 0.1 g AcH/l. The IC–FI method afforded a low relative standard deviation (3.6%) and was free from interferences. The IC–FI procedure was applied to the determination of acetate in mustard sauce and the results obtained agree with those provided by the reference method. Additionally, the developed method permits the simultaneous chromatographic analysis of other anions such as chloride, nitrate, phosphate, sulphate and so on.

RESUMO. Desenvolveu–se um método para determinação de Acetato utilizando separação por cromatografia ionica e detecção espectrofotométrica com injeção de fluxo baseada na reação $La(OH)_3$–I_2. Otimizou–se as condições cromatográficas (eluentes, relação de fluxo, sensibilidade etc) e as variáveis de FI–injeção de fluxo em (longitudes dos tubos, injeção de volume, pH,...). Observou–se uma linealidade sobre a concentração em uma faixa de 0 a 1 gl^{-1} com solução padrão HAc (r = 0,9916) e um limite de detecção de 0,1 g AcH/l. O método IC–FI aportou uma desvio padrão relativamente baixo (3,6) e livre de interferências. O procedimiento IC–FI foi aplicado na determinção de Acetato em mostardas e os resultados obtidos concordam com o método de referência. Além disso o desenvolvimento do método permite a análise cromatográfica simultânea de outros anións tais como cloro, nitrato, fosfato, sulfato, etc.

KEYWORDS: acetate, flow–injection, ion chromatography, UV/V detection, foods.

DOI: 10.48141/SBJCHEM.v3.n3.1995.12_1995.pdf

INTRODUCTION

Ion chromatography (IC) is a sensitive, and versatile analytical technique, useful for the determination of ions. From its birth[1] it has been applied to solve a great number of problems[2,3] in several fields such as clinical, food, industrial and environmental analysis with satisfactory results.

The lack of selectivity and/or sensitivity of most of the detectors is one of the handicaps of this technique. The conductivity detector shows a good sensitivity, though its selectivity is nearly null.

The hyphenation of analytical techniques has proved to be very useful to solve this kind of problems[4]. Generally, chromatographic techniques with post-column derivation[5,6] are used, because it combines a methodology with a great separation ability with high performance detectors.

The coupling of both the separation and identification stages and the automation of the analytical process can be carried out by the FI technique[7,8]. Despite of its potential, the advantages of IC-FIA combination applied to the development of analytical methodologies have not been sufficiently exploited[9].

The determination of acetate is a major difficulty in the analysis of a great deal of industrial, food, fermented and natural products, as it is -in many cases- a basic anion for manufacturing or conservation. For this reason, a lot of methods have been developed making use of techniques such as GC[10] or HPLC[11,12].

The acetate appraising in complex samples requires the use of separation techniques, especially if ionic components such as chlorides, sulphates, nitrates or phosphates are to be simultaneously analysed.

The aim of our work is to test the suitability of IC-UV/V coupled with FI for the automatic analysis of acetate in mustard sauce. This could mean a considerable increase in the versatility and selectivity of the technique, with hardly any sample pre-treatment.

EXPERIMENTAL PROCEDURE

Materials and Equipment

The determinations were carried out in a Dionex 2000 i/sp ion chromatograph equipped with the following columns: guard HPIC-AG4A, analytical HPIC-AS4A and supressor micromembrane AMMS-1, a conductivity detector and Dionex 4290 integrator. The flow injection system consists of a Gilson Minipuls-3 peristaltic pump, a Rheodyne 5701 4-way pneumatic injection valve, a thermostatic water bath and a Pye-Unicam 8625 UV/V spectrophotometer, model 8625 equipped with a Hellma QS 1000 flow-cell (inner volume 18 μL).

Sample management and data adquisition were carried out by a personal computer. Communication software was programmed in C language[13]. The interface used was a PCL-712 PC-LabCard.

- FI Reagents

A 0.1 M $La(NO_3)_3.6H_2O$ solution was used. A 0.01 M aqueous solution I_3^- was prepared by dissolving 1.27 g of I_2 and 4 g of KI made up to a final volume of 1 l with distilled water; this solution was standardised with As_2O_3 and starch as indicator. The $NH_3/Na_2H_2Y/Ca^{2+}$ (Y=EDTA) aqueous solution was prepared by dissolving 1.54 mL of

NH_4OH conc., 0.372 g of $Na_2EDTA.2H_2O$ (ethylenediaminetetraacetic acid disodium salt dihydrated) and 1.64 g of $Ca(NO_3)_2$ in 100 mL of distilled water. This solution is 0.23 M NH_3, 0.01 M Na_2H_2Y and 0.1 M $Ca(NO_3)_2$. 1 M aqueous solutions of Mg^{2+}, Ca^{2+}, Sr^{2+},and Ba^{2+} were prepared from their nitrate salts.

- IC Reagents

Regenerant: H_2SO_4 0.025M. Eluants: sodium tetraborate 0.005M prepared by dissolving 7.628 g of $Na_2B_4O_7.10\ H_2O$ in 4 l of distilled-deionized water. Ten different CO_3^{2-}/HCO_3^- rates were prepared in water (Table IV).

- Other reagents

Pure ethylene glycol (d= 1.11 g l^{-1}); 10% aqueous solution of hydroxilamine-hidrochloride; 0.074 M $FeCl_3$ solution in a 0.37 M H_2SO_4 medium; 1% solution of N-N'dicyclohexyl-carbodiimide in methanol; 1:1 solution of Cl-hydroxilamine 10% and 2.5M NaOH in methanol; 1 M Fe^{3+} solution obtained from iron in concentrated perchloric acid (70%), and 0.1 M $AgNO_3$ were also used.

- Standards

Artificial matrix: Aqueous solution containing 100 mL of 96% ethanol, 0.5 mL of concentrated sulphuric acid, 6 g of sodium chloride, 7 g of citric acid, 2 g of glycerol, 3.8 g of tartaric acid, 3 g of glucose and 0.88 mL of concentrated phosphoric acid per liter.

Acetate standards: prepared by adding acetic acid (density 1.05 g mL^{-1}) to different solvents, namely: distilled water, distilled deionised water, and artificial matrix (diluted 1:5 with distilled deionised water) at different concentrations (0.1, 0.3, 0.5, 0.7 and 1.0 g l^{-1}). All reagents used were of analytical grade.

Sample pretreatment

To obtain the mustard sauce extract the procedure proposed by Canalé-Gutiérrez et al.[14] was followed with some modifications. 5 g of mustard sauce are added to 25 mL of ethanol. The mixture is then stirred and centrifuged. Afterwards, the remaining solution is diluted 1:5 with distilled deionised water.

RESULTS AND DISCUSSION

FI method

In order to develop the FI method, different reactions[15] have been tested in batch for the determination of acetate:

a) Esterification of acids with ethylenglycol followed by the conversion into the corresponding hydroximate and development of a red-violet color with ferric ion. The batch method gave rise to doubtful results with appearance of precipitates and low reproducibility.

b) Esterification with methanol in the presence of N-N'-dicyclohexylcarbodiimide at pH 3 followed by the same steps mentioned above. Batch tests showed the appearance of precipitates which were redissolved by adding Cl-hydroxilamine and sodium hydroxide. However, the complex formed was unstable and the absorbance heavily decreased yielding unacceptable results.

c) Some substances such as lanthanum acetate give a blue color with iodine[16] when they occur in a colloidal state. Consequently, the addition of lanthanum nitrate in hot ammoniacal medium was assayed, a blue adsorption compound being then formed with triiodide. This reaction[17] is extremely sensitive for acetate: 50 μg Ac^- and a concentration limit of 1 in 2000. Sulphate and phosphate interfere, but this can be avoided by precipitating them with barium nitrate. On the other hand, Cl^- hinders the formation of the colloidal acetate product; this is not cited in the handled literature.

From the batch tests performed, the lanthanum/acetate reaction was selected. The UV/V spectrum of the reaction complex showed a maximum absorption at 640 nm, which was adopted as the monitoring wavelength. A FI manifold was then designed in order to automatise the procedure (Figure 1).

Optimisation of the FI variables

In all cases optimisation of the variables was carried out according to the univariate method[18]. The influence of La^{3+}, I_3^- and NH_3 was studied. The precipitation of $La(OH)_3$ in an alkaline medium may be avoided by adding Na_2H_2Y. The great stability of the La^{3+}/EDTA complex hinders the formation of precipitates, but dramatically decreases the analytical signal. The addition of a cation which also forms a complex with EDTA in alkaline medium turned out to be a satisfactory solution to control the concentration of free lanthanum, thus increasing the signal.

Therefore Mg^{2+}, Ca^{2+}, Ba^{2+} and Sr^{2+} were assayed as stabilising cations of the analytical reaction. For this purpose, 1M stock solutions of the corresponding nitrates were used; other salts such as sulphates, phosphates or chlorides interfere in the further reaction. The working concentrations of the assayed cations were chosen according to the solubility constants of their hydroxides, stability of their complexes and pH of work[19]. In all instances, a 0.12 M concentration was adopted except for Mg^{2+}, due to the insolubility of its hydroxide. The best results were obtained with Ca^{2+} (Table I).

In order to check that the signal obtained was not a consequence of the formation of a calcium/acetate complex[20], an assay without lanthanum was carried out, using aqueous standards of up to 1 g l^{-1} of HAc. Under the experimental conditions no formation of this complex was observed.

Table I

Behaviour of different cations with respect to the formation of the Lanthanum–EDTA complex*

HAc (g l^{-1})	Mg^{2+} 0.01 M	Ca^{2+} 0.12 M	Sr^{2+} 0.12 M	Ba^{2+} 0.12 M
0	0.065	0.943	0.898	0.881
0.5	0.050	0.881	0.838	0.824
1.0	0.040	0.785	0.768	0.765

* Results in absorbances

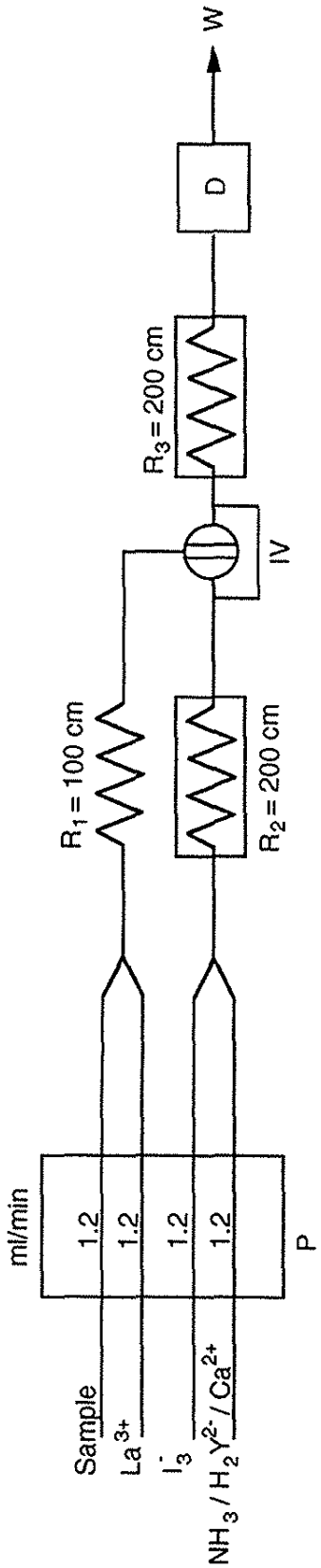

Figure 1. FI manifold for the spectrophotometric determination of acetate. P– Pump, R_1, R_2, R_3– length of the reaction coils (R_2 and R_3 thermostatized at 30 °C); IV– Injection valve; D– Detector; W– waste.

Then the influence of the temperature (over the range 15–50°C) was studied. At higher temperatures, the reaction takes place faster, but the signal remains constant. Additionally, bubbles from the release of ammonia appear. Therefore, a very moderate temperature (30°C) turns out to be enough for a sufficiently rapid development of the reaction, which is not possible with temperatures under this value. On the other hand, the alternative of increasing the residence time is not satisfactory, since a precipitate appears as a consequence of prolonging the contact time between the carrier and the sample. For this reason, a temperature of 30 °C was selected in this work.

The pH influence was also studied in the range between 5.5 and 12.5 The optimum pH value –achieved by adding NH_3– turned out to be 11. An excess of the reagent produces a precipitate, whereas with pH values below 5.5 there is scarcely any reaction. Table II shows the concentration ranges studied for each of reagent (La^{3+}, I_3^-, NH_3, H_2Y^{2-} and Ca^{2+}), as well as their optimal values.

The studied FI variables together with the results obtained are summarised in Table II. Injection volumes higher than 200 μL give rise to undesirable double peaks,

Table II
Results of the optimisation of FI variables

	Range	Optimum
La^{3+} (M)	0.005–0.1	0.01
I_3^- (M)	0.0005–0.05	0.001
NH_3 (M)	0.005–2	0.23
H_2Y^{2-} (M)	0.006–0.02	0.01
Ca^{2+} (M)	0.04–0.14	0.1
pH	5.5–12.5	11
T (°C)	15–50	30
Injected Vol. (μL)	14–260	43
Flow-rate (mL min^{-1})	0.6–2	1.2
R_1 (cm)	50–200	100
R_2 (cm)	50–300	200
R_3 (cm)	50–300	200

whereas values under 20 μL do not yield detectable signals. A volume of 43 μL was used in this work.

The most recommendable flow-rate was 1.2 mL min^{-1}, which produces a sufficient sensitivity as well as a good sampling frequency (80 h^{-1}).

Coil lengths also play a role in the performance of the method. R_1 values over 100 cm result in the appearance of precipitates and double peaks. As for R_2 and R_3, length values of 200 cm are recommended so that the reagent becomes warm and homogeneous with the carrier without precipitating.

Features of the FI method

With the optimum values of the variables, a calibration graph was run with HAc standards (0.1–1 g l^{-1}). The equation obtained was Abs= 381.7 – 159.8(Ac^-) (r= 0.9996) and the precision of the method, evaluated on 11 samples of 0.5 g l^{-1} injected in triplicate, was 0.96% RSD.

The validity of the method was studied by comparing it with the distillation reference method[21]. Water and artificial matrix spiked with acetic acid standard are used in both cases. The results obtained (Table III) show that the automatic method is an excellent alternative to the manual procedure.

The influence of the chromatographic eluants (HCO_3^-/CO_3^{2-} 1.7 mM/1.8 mM and sodium tetraborate 0.005 M) was evaluated by adding different HAc concentrations (0, 0.5 and 1 g l^{-1}). No interferent effects were observed.

Table III

Comparison between the results obtained in both the reference and the FI methods* for Ac^- determination

HAc added	Water		Artificial Matrix	
	Reference	FI	Reference	FI
0	0.01	0.02	0.02	0.02
0.1	0.12	0.09	0.10	0.09
0.3	0.26	0.30	0.31	0.29
0.5	0.48	0.49	0.49	0.50
0.7	0.69	0.70	0.72	0.72
1.0	0.99	1.00	1.01	0.99

* Concentrations in g l^{-1}

Optimisation of the IC system

Once the FI method was set up, the IC system was then optimised. The working medium was an artificial matrix at concentrations of acetic acid over the range 0.1–1 g l^{-1}. This matrix contains glucose, ethanol, citric acid, glycerol, sodium chloride, tartaric acid, phosphoric acid and sulphuric acid, with the purpose of applying the methodology to real samples. Chloride, though an interfering anion in the spectrophotometric determination, is added since it is common in any type of sample.

Firstly the following working conditions were adopted. Eluant: sodium carbonate 1.8 mM / sodium bicarbonate 1.7 mM, flow–rate 1 mL min^{-1}. Regenerant: sulphuric acid 0.025 M. Flow–rate: 6 mL min^{-1}. Pressure: 44 bar .Sensitivity: 30 μS .Injected sample volume: 50 μL. Under these conditions, the resolution of the chromatogram obtained was not satisfactory and ought to be optimised.

Study of IC variables

Several dilutions of the artificial matrix with distilled deionised water were assayed (1:2, 1:5, 1:10 and 1:50). The best chromatographic results of resolution and reproducibility were obtained with a 1:5 dilution and a pressure of 44 bar.

In order to optimize the sensitivity of the detector, several chromatograms were carried out by injecting the artificial matrix (diluted 1:5) containing 0.5 g l^{-1} of HAc at sensitivities ranging from 10 μS to 1KS. The optimum value turned out to be 300 μS and was used for subsequent assays.

The optimisation of the eluant concentration was carried out using Na_2CO_3/$NaHCO_3$ and varying the rate carbonate/bicarbonate. For this purpose, 1 g l^{-1} of HAc was added to the artificial matrix diluted 1:5 with distilled deionised water. The results obtained are shown in Table IV. It can be noticed that, for a constant concentration of carbonate and an increasing concentration of bicarbonate (eluant 1 to 6), the chromatogram scarcely changes; there is only a slight increase in the area as well as in the retention time of the monovalent anions. On the other hand, if the concentration of bicarbonate decreases, the retention time of the first peaks increases (unlike their width) and so does the area. Additionally, the retention time of sulphates, phosphates and so on decreases and their resolution improves.

If the concentration of bicarbonate remains constant while that of carbonate decreases (eluant 7 to 10), retention times increase and peaks become wider. However, peaks which appear close to acetate can be integrated, at the cost of increasing the retention times of divalent ions up to non–recommendable 20 minutes. The chromatogram can be shortened if the concentration of carbonate is increased up to 2.4 mM (while keeping constant the concentration of bicarbonate), but Ac^- and Cl^- anions clearly overlap and cannot be separated.

The identification of the peak corresponding to the acetate ion was carried out in eluant number 9, by diluting the artificial matrix (1:5) with water and later adding 10 g l^{-1} of acetic acid (amount in excess with the purpose of heavily increasing the chromatographic peak of acetate). Its signal corresponds to a retention time –under the working conditions– of 1.94 min. Figure 2 shows a typical chromatogram obtained with the optimized system.

Table IV
Study of the resolution of the chromatograms according to the eluant[a]

		Monovalent anions[b]		Divalent anions[c]	
Eluant No.	$[CO_3^{2-}]/[HCO_3^-]$ (mM)	Resolution	RT (min)	Resolution	RT (min)
1	1.8/1.7	No	3,09	Yes	-- 7,19 8,44
2	1.8/1.6	No	3,13	Yes	6,34 7,33 9,45
3	1.8/1.8	No	3,13	Yes	6,14
4	1.8/1.4	No	3,27	Yes	-- 7,03 8,98
5	1.8/1.0	Yes	0,86 3,34	Yes	5,99 6,90 8,93
6	1.8/0.7	No	3,44	Yes	5,90 6,97 8,97
7	1.4/1.7	No	3,29	Yes	7,37 8,13 10,44
8	1.0/1.7	Yes	1,95 3,35	Yes	9,41 10,81 11,33 12,39
9	0.6/1.7	Yes	1,94 3,65	Yes	13,37 15,32 16,52 18,36
10	2.4/1.7	No	2,88	Yes	5,179 6,06 6,94

a) Sample: artificial matrix; b) Ac^- and Cl^-; c) P: Phosphate, S: Sulphate, T: Tartrate and C: Citrate.

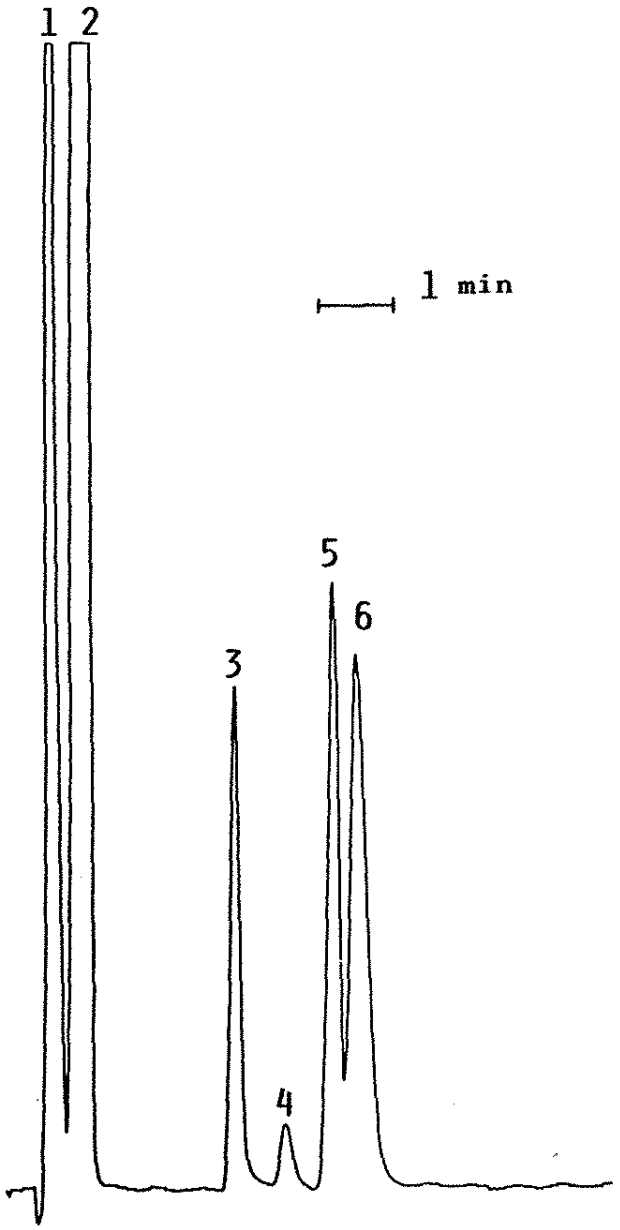

Figure 2. Chromatogram of artificial matrix, diluted 1:5. Eluant number 9 , flow rate 0.8 mL min^{-1}. Regenerant H_2SO_4 0.025 M, flow rate 6 mL min^{-1}. Conductivity detector at 300 μS. 1. Ac^- (RT 2.66, Area 83485605); 2. Cl^- (4.39; 149909639); 3. Phosphate (13.05; 27721055); 4. Sulphate (16.10; 4652336); 5. Tartrate (18.69; 40284386); 6. Citrate (20.04; 55960331).

Features of the IC method

Under the optimum conditions, a calibration graph was run with HAc standards in distilled deionised water at concentrations between 0.1 and 1.0 g l^{-1}. The equation obtained was: Area = 1.25 + 2.64 (HAc) (r= 0.9970). These results show that the methodology is applicable to the determination of acetate in the working medium.

The reproducibility, evaluated on eleven samples of 0.5 g l^{-1} of HAc in the same medium (artificial matrix diluted 1:5) was 2.03 % RSD, which is acceptable.

IC–FI coupling

The configuration proposed is shown in Figure 3. The hyphenated system is designed in such a way that the chromatograph acts as a separating agent of the acetate from the other compounds of the sample, whereas the FI system acts like a selective spectrophotometric detector for acetate. When both systems are coupled, it is very important to isolate the sample plug containing this anion from the eluate. The location of the injection valve in the FI system plays a key role. Its optimum place is right after the conductivity cell of the chromatographic detector. The automatic injection valve manages to capture the volume containing the Ac^- anion by means of the computer. At a constant flow–rate, the computer makes the valve load at a certain time, corresponding to the retention time of Ac^- and it remains open for an established period.

The flow–rate of the FI system should be adapted to that of the chromatograph, so that there is no internal overpressure which could decrease the flow of the reagents with the subsequent appearance of a precipitate of $La(OH)_3$. These different flow–rates could also be responsible for retentions and high pressures in the chromatographic system. Therefore, once the system is set in motion, the flow–rate of the chromatograph must be measured and adjusted to the optimised value (see above). The best value is 1.25 mL min^{-1}. The flow–rate of the FI system equals that of the chromatograph by adjusting the pump speed; in that way, the initial condition of the method does not suffer important modifications.

The sample volume injected in the IC exerts a major influence on the response given by the FI system. The loops used in the chromatograph and the FI system as well as the reactor lengths are optimised by carrying out assays with HAc standards (concentrations 0, 0.5 and 1.0 g l^{-1}) in distilled deionised water. The results are shown in Table V.

The sensitivity of the chromatograph must be adapted to the conductivity of each analyte according to the concentration of the samples. The sensitivity was then optimised for each type of injected sample; it turned out to be 300 μS in all instances.

Table V

Results of the optimisation of the IC–FI variables for Ac^- determination

	Pressure (bar)	Injected Vol. IC (μL)	Injected Vol. FI (μL)	R_1 (cm)
Range	27–50	50–200	14–200	0–200
Optimum	45	150	60	0*

* 86 cm minimal distance between the IV position and the detector (Fig. 3)

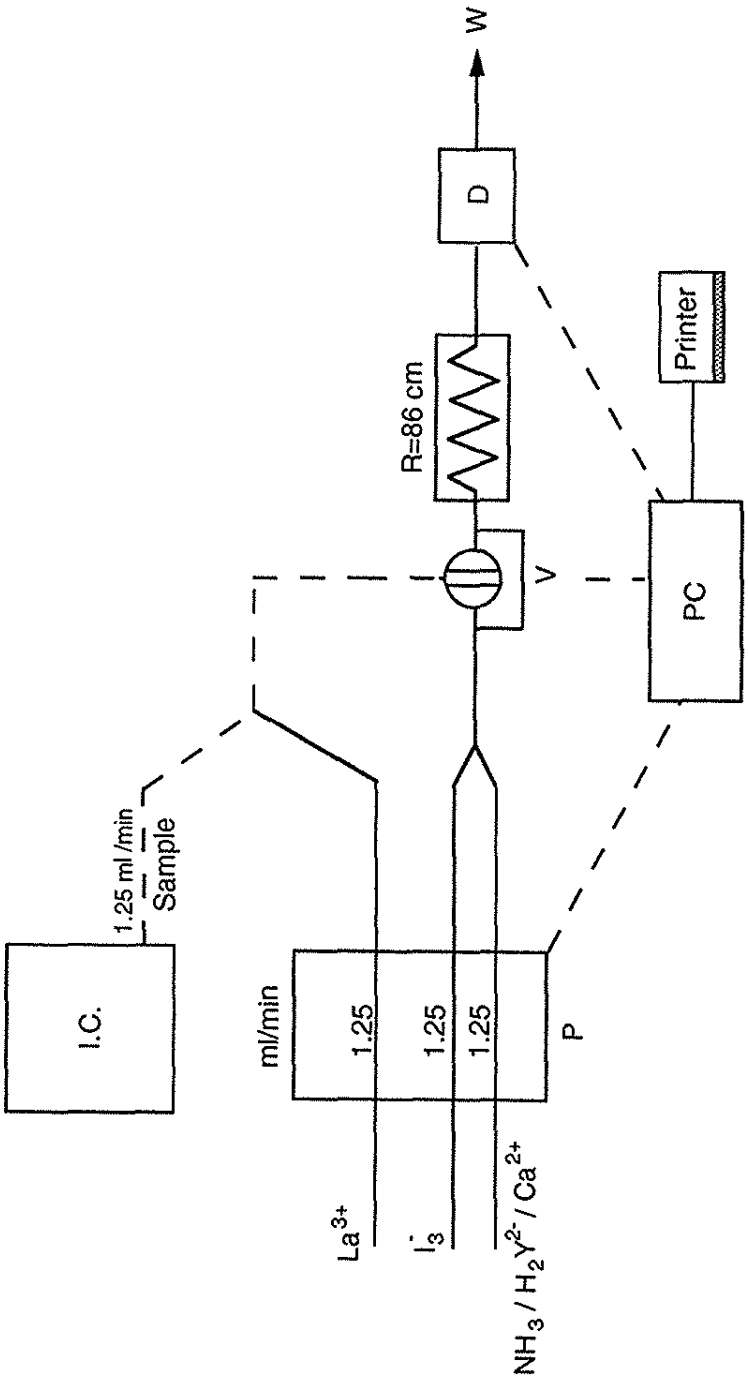

Figure 3. IC–FI configuration for the determination of acetate.
IC– ionic chromatograph; P– pump; V– automatic injection valve; PC– computer; R– reaction coil (thermostated at 30 °C); p– printer; D– detector; W– waste.

The optimised $Na_2CO_3/NaHCO_3$ solution was used as chromatographic eluant and this does not offer any problem inside the FI system, as it is only a carrier.

In order to determine the concentration of acetate, a calibration graph was run with HAc standards in the artificial matrix diluted 1:5 with distilled deionised water. These standards covered the range between 0 and 1 g l^{-1}. The result in absorbance and area was: Abs = 0.704 - 0.117(HAc) (r=0.9916).

Application to real samples

The proposed method was applied to the determination of acetate in mustard sauce. 0.6 mM CO_3^{2-}/1.7 mM HCO_3^- was used as eluant and the extracts were diluted 1:5 with distilled deionised water.

According to the developed method, the concentration of acetate was evaluated by means of the post-column coupled FI method.

The results obtained with both the proposed and the reference method are very similar. The mean values for mustard sauce obtained by FI-IC and the distillation reference method are 23.6 and 23.3 g K^{-1}, respectively with a recovery of 98.7%. Furthermore, the chromatographic method allows for the simultaneous analysis of other anions which are present in the samples such as nitrate, phosphate, sulphate and so on. The usefulness of the method is then demonstrated.

CONCLUSIONS

The IC-FI coupling widens the application field of IC. The specificity in the analysis of ionic species increases, as well as the resolution of mixtures of ions which co-elute. Its application to real samples has been satisfactory, although there is still much to do with matrices other than those assayed.

With this method, acetate can be determined in complex samples in the presence of other ionic compounds.

The IC-FI coupling permits the use of an identification reaction for acetate (theoretically unspecific) with good results in the presence of potentially interfering anions such as Cl^-.

The advantages of this method are: simultaneous determination of several anions; detection limits of mg l^{-1}; reduction of the time of analysis to minutes; minimum sample preparation; use of unexpensive reagents at very low concentrations and easy analysis of anions in complex matrices.

Acknowledgements. This research was supported by the CICYT, project ALI 90/0633. The authors acknowledge the financial aid.

REFERENCES

1. H. Small, T.S. Stevens and W.C. Bauman, *Anal. Chem., 47*,1801 (1975).
2. D.T. Gjerde and J.S. Frith, "*Ion Chromatography*", 2nd edition, Hüthing, Heilderberg, (1987).
3. R.E. Smith, "*Ion Chromatography Applications*", CRC Press, Boca Raton, (1988).
4. O.F.X. Donard and F.M. Martin, *TrAC, 11*, 17 (1992).
5. G. Redant, *TrAC, 11,* X (1992).
6. I.S. Krull, "*Reaction Detection in Liquid Chromatography*", Marcel Dekker, New York, (1986).
7. M.D. Luque de Castro and M. Valcárcel, *J. Chromatogr., 600*, 183 (1992).
8. H. Engelhardt, R. Klinkner and G. Schöndorf, *J. Chromatogr., 535*, 41 (1990).
9. O.N. Obrezkov, V.I. Shlyamin, O.A. Shpigum and Yu.A. Zolotov, *Anal. Sci., 7*, 321 (1991).
10. S.A. Kupina, J.L. Kutchnski, R.D. Williams and R.T. De Soto, *Am. J. Enol. Vitic., 33*, 67 (1982).
11. A. Schneider, V. Gerbi and M. Redoglia, *Am. J. Enol. Vitic., 38*, 151 (1987).
12. V. Heidger, *Weinwirtschaft–Technik, 126*, 12 (1990).
13. S. Pro–Labrandero, "*Desarrollo de un sistema de adquisición de datos y control para su utilización en métodos analíticos en flujo (FIA)*". Proyecto Fin de Carrera. E.T.S.I.I. Universidad Politécnica de Valencia. Valencia. (1993), 300pp.
14. L. Canalé–Gutiérrez, A. Maquieira and R. Puchades, *Analyst, 115*, 1243 (1990).
15. M. Pesez and J. Bartos, "*Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs*", Marcel Dekker, New York, (1974).
16. F. Pino–Pérez and M. Valcárcel–Cases, "*Equilibrios iónicos en disolución. Análisis volumétrico*", Urmo S.A. Ediciones, Bilbao, (1978).
17. A.I. Vogel, "*Vogel's textbook of macro and semimicro qualitative inorganic analysis*", 5th edition, Longman, New York, (1979).
18. D.L. Massart and A. Dijkstra, "*Evaluation and Optimization of Laboratory Methods and Analytical Procedures*", Part 2, Elsevier, New York, (1988).
19. F. Bermejo–Martínez, in: "*Chelates in Analytical Chemistry*", H.A. Flaschka, A.J.Jr. Barnard (Ed.), Vol.5, Part D, Marcel Dekker, New York, (1976).
20. F.A. Cotton and G. Wilkinson, "*Advanced Inorganic Chemistry*", 4th edition, John Wiley and Sons, New York, (1980).
21. J. Ribéreau–Gayon, E. Peynaud, P. Sudraud and P. Ribéreau–Gayon, "*Traité d'oenologie. Sciences et techniques du vin*". Tome 1, Dunod, Paris, (1976).

The SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY (ISSN: 2674-6891; 0104-5431) is an open-access journal since 1993. Journal DOI: 10.48141/SBJCHEM.
http://www.sbjchem.com.
This text was introduced in this file in 2021 for compliance reasons.